CLAIM + DETAILED DESCRIPTION

[Claim(s)]

[Claim 1]A process of harvesting algae of an a)-fucus (Pheophyceae) family;

b) - A process to wash;

c) - A process which carries out spallation and minute spallation;

A process of removing a cell spall by d)-centrifugality;

A process which carries out the fraction by adsorption of a up to [precipitation using e)-acid, and activated charcoal];

An educt which is rich in betaine of algae obtaining using a procedure including a filtering step controlled by a quantitative analysis of glycine betaine using f)-HPLC.

[Claim 2]In a process of this procedure, algae are Laminaria. The educt according to claim 1 which is OKUROROIKA (Laminaria ochroleuca).

[Claim 3]The educt according to claim 1 whose acid is hydrochloric acid in e process of this procedure.

[Claim 4]f) The educt according to claim 1 which is the tangential filtration (tangential filtration) whose filtration has a cut-off threshold (cut-off threshold) of 1000D in a process.

[Claim 5]The educt according to claim 1 by which glycine betaine being included at least 10%.

[Claim 6]Use of the alga extract according to any one of claims 1 to 5 as an active ingredient in a constituent of cosmetics or a physic use.

[Claim 7]The use according to claim 6 as an osmosis protection active ingredient.

[Claim 8]The use according to claim 6 as an anti--radical active ingredient.

[Claim 9]The use according to claim 6 as an anti--aging active ingredient of the skin.

[Claim 10]Cosmetics or a medicinal composition cream, gell, emulsion, or emulsion (milk) type containing the educt according to any one of claims 1 to 5 1 to 40%.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the use as an active ingredient in the alga extract which is rich in the betaine, its extraction method, cosmetics, and a medicinal composition.

[0002]

[Description of the Prior Art]It is the eternal purpose of the manufacturer of cosmetics to provide especially the constituent by having contacted sea water for a long time, or having been exposed to the sun under more nearly special environment, for contamination, temperature variation, and humidity change, which ensures protection of the skin mainly from the active influence of outside environment.

[0003]It searches for various active ingredients in desirable natural vegetables or animal origin. Some molecules contributed to cell protection against various kinds of conditions of stress are actually found out naturally. Playing the role which the betaine maintains homeostasis and protects intracellular environment from pervious stress especially in these is known (especially). Biochem. J. 282, Petronini et al., 1992, 69-73 and Sutherland and others, J. Bacteriol., 168, 1986, and 805-814 Reference.

[0004]In a bacterium and plankton, these molecules are found out in the both sides of an animal and the plant kingdom.

[0005]In the case of the algae which receive by turns the contact with the algae which receive change of osmotic pressure, especially a wave, and an appearance, there are they abundantly.

[0006]This is a Reason for having used the brown algae of a lot of fucus (Pheophyceae) families on the seashore in Brittany, in order that these people may look for the molecule which is potentially [especially for cosmetic industry] interested.

[0007]

[Means for solving problem]Especially these people are Laminaria. It tried hard enriching this educt by the substance of a betaine family using an easy procedure to perform on preparing the educt of the algae known as OKURORIKA (Laminaria ochroleuca), and an industrial scale.

[0008]The procedure of this invention is a. - Process of harvesting fresh algae;

Process of washing b)-this algae;

c) - Process which carries out spallation and minute spallation;

Process of removing a cell spall by d)-centrifugality;

Process which carries out the fraction by adsorption of a up to [e)-acid, precipitation using HCl preferably, and activated charcoal];

The filtering step stopped when it is monitored from the quantitative analysis of the glycine betaine using f)-HPLC and the glycine betaine concentration of filtrate reaches to 12.5% () [this filtration] **** is preferably included by the tangential filtration (tangential filtration) with the cutoff threshold of 1000D.

[0009]
[Mode for carrying out the invention]It is standardized about the glycine betaine concentration which shows at least 10%, and the educt obtained in this way will be called lamina yne (Laminaine) since the source of a source material is a Fucus-vesiculosus group (Laminaria).
[0010]In addition to the publicly known character of the betaine as an osmosis protecting agent, these people clarified anti--radical effect and the stimulative effect about new synthesis (neosynthesis) of a cell membrane and the component of an extracellular matrix (proteoglycans and glycosaminoglycans). Especially these people planned the use as an active ingredient of the constituent of a cosmetics use for the alga extract of this invention, and used these character that cannot be predicted.
[0011]Although the character from which the alga extract differed is clarified by these people and this becomes clear from the embodiment shown below, In order to improve the condition of the skin in fact, and in order [and/or it becomes a cause of free radical discharge,] to improve the resistance over various condition of the stress which slows down skin regeneration and promotes aging, it can expect using especially the educt of this invention.
[0012]Thus, especially this invention relates to using the above-mentioned alga extract as osmosis protection, anti-- radical, and an active ingredient of anti-skin aging in a cosmetic composition or a medicinal composition.
[0013]This active ingredient can be used in order to mix to what kind of cosmetic composition and medicinal composition cream, gell, the emulsion, or emulsion (milk) type.
[0014]This invention relates to the cosmetic composition or medicinal composition which contains the above-mentioned alga extract 1 to 40% again.
[0015]The same active ingredient may be extracted from other plants using an extraction method similar to the procedure shown below.
[0016]The purpose of this invention spreads in the use as an anti-aging treatment of the skin as an active ingredient which protects the above-mentioned active ingredient extracted from the arbitrary plants containing it again against a free radical.
[0017]The following embodiments explain this invention, without limiting the range of this invention.

[0018]Embodiment 1: Extraction process Laminaria OKURORIKA Alga (Laminaria ochroleuca alga) is the northern part seashore of Brittany (Brittany), and was harvested in September after May.
[0019]The harvested algae are moved all over the sea water in a container. They are processed shortly after arriving at a manufacture site.
[0020]In the blade crusher of a URUSHIERU (Urschell, registered trademark) mold, they are crushed and are made into the particles of the size of the range of 100-200 micrometers.
[0021]In glycol content (50%) deionized water, the crushed material is a rate of the spallation thing

430g per 1 l. of solution, and is suspended.

[0022]Since more detailed spallation makes the content of a cell emit, the blade homogenizer of a URUTORACHU Lux (Ultra Turax, registered trademark) mold is used, and it is performed at ordinary temperature for 3 hours.

[0023]The crushed material was centrifuged for 45 minutes at 16000 g, in order to remove a cell spall. Supernatants are collected and vacuum concentration is carried out 1/3 of the original capacity.

[0024]Subsequently, it diluted to 1/20 in distilled water, in order to set pH to 1.0 at it, HCl and 4% of activated charcoal were added 35%, and churning by the magnetic stirrer during 30 minutes was presented.

[0025]Being able to settle [and] the substance (especially protein) which is not desirable as for a large number by addition of acid, activated charcoal adsorbs various organic substances (phenol type substance etc.).

[0026]Subsequently, the supernatant condenses betaine by carrying out tangential filtration (tagential filtration) with the cut-off threshold (cut-off threshold) of 1000D. Clear filtrate was collected and it analyzed using the HPLC chromatography provided with the UV detector in accordance with the procedure (J. Agricultural and Food Chemistry, 45, 3:774-776) of Zamarreno.

[0027]As a standard, using 0.2 of the refined glycine betaine (sigma company), 0.4, 0.8, and 2weight % of distilled water solution, it dilutes 20 times and a calibration curve is prepared.

[0028]An alga extract sample is measured in the surface of the chromatography peak as compared with a calibration curve.

[0029]The concentration step stopped, when glycine betaine concentration reached to 12.5**2.5%. The educt obtained in this way is called "lamina yne (Laminaine)" in the following examples.

[0030]Embodiment 2: The fact that the proof lamina yne of the anti radical effect had the anti radical effect was proved by analyzing quantitatively the malondialdehyde in the fibrocyte of the Homo sapiens dermis cultivated from the skin biopsy. An examination is done about the culture between the fourth-generation cultivation from the second generation.

[0031]Malondialdehyde (MDA) quantitative analysis: Lipid-peroxidation index (Lipoperoxidationindex).

[0032]fibrocyte -- a large number -- multi--- a well -- into 1 ml of RPMI 1640 cultivation culture media supplemented with L-glutamine of 10% fetal calf serum and 10mM, and the gentamicin of 80microl, it is a rate of the cell of 10^5 ** per well, and was distributed at the dish (24 wells each). They ranked second and were maintained in the CO_2 incubator for 24 hours.

[0033]lamina yne is a rate of three wells per one dose -- multi--- a well -- it was distributed by concentration (a pure article, 1/2, 1/5, and 1/0) which is different in a dish. :-3 well on which various examinations were done in parallel received only the solvent.;

-Three wells received SOD (super OKISHIDODISU mutase) and the catalase.; (negative control)

-Three wells received the xanthine hypoxanthine complex.; (examination of the efficiency of defense enzyme (SOD+ catalase))
-In addition to the xanthine hypoxanthine, 12 wells received four sorts of dilution of lamina yne.;
-Twelve wells received four sorts of dilution of lamina yne.
[0034]50mM TRIS buffer of :-250microl suspended in the following in the cell pellet after the trypsinization (trypsinisation) of the extraction cell of malondialdehyde, and centrifugal separation, pH 8, NaCl content of 0.1M;
-HCl of 7%SDS-300microl of 20mM EDTA-25microl (0.1N)
-It cooled in ice water after a 1-hour incubation in the dark place of 50 ** of 0.67% thiobarbituric acid solution of 1% phosphotungstic-acid solution-300microl of 38microl, and n-butanol of 300microl was added to each tube. These were centrifuged for 10 minutes at 0 ** and 100,000 g. Upper phases were collected for the MDA quantitative analysis.
[0035]The malondialdehyde quantitative analysis MDA was quantitatively analyzed by measuring fluorescence after separating a MDA-TBA complex using following HPLC.
[0036]- A model 2.200 BISHOFU (Bischoff) pump model ARUKOTTO (Alcott) autosampler automatic injector C18 ultra SEPPU (Ultrasep) column (30 cm x 0.18 cm), A porosity [of 6 micrometers] (porosity)-fluorescence detector, JUSCO (Jasco)821-F1.
[0037]Fluorescence detection was performed by 515 nm of excitation, and 553 nm of photogenesis. The used eluent consisted of methanol:water and 40:60 (v/v), and the pH was adjusted to 8.3 by KOH of 1M.
[0038]a quantum -- an ICS software package (pick (Pic)3) (the instrumentation.) It was carried out by making it connected with the standard sample (0.125;0.25;0.5;1microM) processed similarly using KONSOMMA bull service (Instrumentation, Consommable Service).
[0039]A quantitative analysis of protein quantitative-analysis protein was carried out using the Bradford (BRADFORD) method. An increase with an extinction of 595 nm is proportional to concentration of protein measured using uni-cam (UNICAM)8625 spectrophotometer.
[0040]The quantitative analysis 1 of malondialdehyde in consequence-cell homogenate - Physiological lipid peroxidation [0041]
[Table 1]

ラミナイン	MDA（μM／mgタンパク）	MDAの減少%
コントロール0	618±58	-
純品	558±19	-5
2／2希釈	556±76	-10
1／5希釈	481±65	-22
1／10希釈	467±69	-24

[0042]In 1/[1/5 -] 10 dilution, remarkable defense to physiological lipid peroxidation was especially accepted under existence of lamina yne.

[0043]2 - For the purpose of guided lipid-peroxidation ********, only 1/[1/5 and] 10 dilution was used from a viewpoint of reduction which is not significant as for physiological MDQ of a lamina yne pure article or 1/2 dilution.

[0044]

[Table 2]

試薬	MDA（μM／mgタンパク）	MDAの減少%
コントロール	656 ± 39	-
Sod-Cat	418 ± 49	-36
Xant-Hypox	1156 ± 174	+76
SOD-Cat-Xant-Hypox	806 ± 71	-30
1/5-Xant-Hypox	804 ± 24	-30
1/10-Xant-Hypox	460 ± 25	-60

[0045]In 1/[1/5 and] 10 dilution to lipid peroxidation from which lamina yne happens by a system which produces a free radical of the xanthine/hypoxanthine, It was admitted giving considerable defense and that it was comparable as defense given by SOD-catalase of publicly known defense enzyme at least.

[0046]Under the used experimental condition, lamina yne has anti radical activity significant on Homo sapiens fibrocyte after contact of 24 hours in the culture medium in this way.

[0047][the fibroblast obtained from the biopsy of the proteoglycans by the fibroblast in an embodiment 3-culture, and the actual proof Homo sapiens skin of a glycosaminoglycan synthesis stimulus] By two to 4 passage, wind into a culture medium, carry out multiplication, and 10% fetal calf serum, It was made to distribute to the dish of 25cm^2 of a large number containing the 10-ml RPMI 1640 cultivation culture medium which added 10mM L-glutamine and the 80 microg [/ml] gentamicin at a rate of 10^5 ** / ml. Then, it maintained among the CO_2 incubator for 24 hours.

[0048]By various concentration (1/2, 1/5, and 1/10 dilution), lamina yne was distributed to each dish based on three dishes per one dose. Simultaneously, only water was added to three dishes and it was used for the purpose of control.

[0049]Pulse technique was adopted, in order to incorporate the radioactive precursor of the cell pretreated in lamina yne and to investigate capability (capacity). - glucosamine which is a radioactive precursor ([^{3}H]) was added into the culture in 18 hours before recovery of a cell. Cell-product contact time was 24 hours at 37 **.

[0050]After suction removed a culture medium, it was made to exfoliate by washing a cell twice by a serum free medium, removing the radioactivity which was not incorporated, and rubbing the surface of a culture from a base material after that. Once again, the cell was washed by the culture medium and the at-long-intervals heart was carried out at 600 g after that for 5 minutes. This cell pellet was followed in the following operations.

[0051]- New synthesis of the quantitative-analysis-total glucosaminoglycan (GAGs) of the

proteoglycans using FPLC (first protein liquid chromatography (Fast Protein Liquid Chromatography)) (neosynthesis)
- Characterization of the glycosaminoglycan by the quantitative-analysis-selective digestion of total protein.
[0052]- The 1st fraction of the extraction film proteoglycans pellet was transposed to suspension in 1M NaCl containing the deoxyribonuclease (50 U/ml) and protease inhibitor. Then, the homogenate was incubated at 4 ** for 2 hours. Centrifugality was carried out for 30 minutes at 12000 g, and the 1st homogenate containing the proteoglycans (peri-membrane) of the circumference of a film was obtained. Proteoglycans were extracted from two sorts of other components using this pellet (C1).
[0053]The 0.1% sodium azide solution which contains sodium deoxycholate 4% was made re-suspended, C1 pellet obtained from the 1st extraction process of film penetration (Trans-membrane) proteoglycans was treated with ultrasonic waves for 20 seconds at 75 mV, and it incubated at 4 ** after that for 2 hours. The second supernatant containing film penetration proteoglycans was obtained by carrying out the at-long-intervals heart at 12000 g for 30 minutes. Proteoglycans were extracted from the matrix compartment using this pellet (C2).
[0054]It transposed to the suspension in 4M HCl guanidine and 50mM sodium-acetate buffer which contains the triton X-100 and protease inhibitor 0.1%, having washed the matrix proteoglycans pellet C2 3 times, and agitating it after that with sodium azide solution, 0.1%.
[0055]The third supernatant containing matrix proteoglycans was obtained by carrying out the at-long-intervals heart at 12000 g for 30 minutes.
[0056]Before refining using the refining FPLC using 2-FPLC, each of three sorts of supernatants was settled at 4 ** among the 3 times as many pure 100% ethanol of capacity all night, and the at-long-intervals heart was carried out at 12000 g after that for 30 minutes. The obtained pellet was made re-suspended in 50 mM Tris-HCl buffer and pH 7.4.
[0057]Three samples were followed in the same analytical protocol.
[0058]Anion-exchange chromatography was used. This was actually promoted by high-density negative **** supplied to proteoglycans with those glycosaminoglycan chains.
[0059]Each pellet was made re-suspended in 250-ml 50 mM Tris-HCl buffer and pH 7.4. K 10/40 column (Pharmacia) was filled with DEAE-Sepharose gel CL-6B (Pharmacia). This anion-exchange gel gives high separability and high yield. ; which poured in 100micro of each sample I -- the soectrofluorometer with a wavelength of 280 nm detected after elution.
[0060]The peak containing proteoglycans was eluted by 1M NaCl.
[0061]3-quantitative-analysis radioactivity measurement It carried out at the outlet from HPLC using the puckered (Packard) (Flo-one) counter.
[0062]In order to determine the character of GAGs which exists in the identification fraction using 4-preferential degradation, a different decomposition reaction was performed.

[0063]The digestive AC chondroitinase by AC chondroitinase carries out depolymerization of the glucuronic acid, and, as a result, decomposes chondroitin sulfate.
[0064]The aliquot of the material which GAGs freeze-dried was incubated in 37 ** of Tris-HCl buffer and pH8 Naka containing AC chondroitinase (0.2U/ml) for 1 hour. It was made to stop by freezing a reaction at -20 **.
[0065]The digestive ABC chondroitinase by ABC chondroitinase carries out depolymerization of glucuronic acid and the iduronic acid, and, as a result, decomposes chondroitin sulfate and the dermatan sulfate.
[0066]The second aliquot of the lyophilization material of GAGs was incubated in 37 ** of same Tris-HCl buffer and pH8 Naka containing ABC chondroitinase (0.5U/ml) for 1 hour. The reaction was stopped at -20 **.
[0067]The digestive nitrous acid solution by nitrous acid was prepared by mixing sodium nitrate (0.148M) and acetic acid (3.6M).
[0068]; which cuts a glycoside linkage from the glucosamine binding in which nitrous acid has an amino sulfonate group by the 2nd place, as a result this are specific to heparin and heparan sulfate.
[0069]; which performed the reaction for 80 minutes with ambient air temperature -- it was stopped by adding 1M ammonium sulfate. Hydrolyzate carried out dry vaporization and was made to re-replace by 50 mM Tris-HCl solution after that for analysis.
[0070]The gel of ****** of GAGs on CL.6B gel (Pharmacia) is suitable for studying the molecule of low molecular weight. It is made to equilibrate by 50 mM Tris-HCl buffer and pH 7.4, and flowed into K 10/40 column (Pharmacia). Elution was performed using the same buffer containing 0.35M NaCl. 1-ml fractions were collected and it counted after adding 5 ml of SHINCHI rating liquid.
[0071]
[Table 3]

放射能測定の結果：プロテオグリカンの新合成における効果

－膜周囲のプロテオグリカン

ラミナイン	Cpm	%取り込み
コントロール0	155 ± 32	-
1/5 に希釈	208 ± 25	34
1/10 に希釈	215 ± 12	38

－膜プロテオグリカン

ラミナイン	Cpm	%取り込み
コントロール0	170 ± 25	-
1/5 に希釈	253 ± 12	48
1/10 に希釈	285 ± 17	67

－マトリクスプロテオグリカン

ラミナイン	Cpm	%取り込み
コントロール0	135 ± 25	-
1/5 に希釈	170 ± 43	25
1/10 に希釈	154 ± 19	14

[0072]Thus, we observe a significant stimulus of new synthesis of the proteoglycans under lamina yne existence.

[0073]

[Table 4]

新合成したグリコサミノグルカンの特徴付けの結果

－デルマタン硫酸及びヘパラン硫酸の新合成（ＤＳ及びＨＳ）

ラミナイン	Cpm	%取り込み
コントロール0	256 ± 24	-
1/5 に希釈	263 ± 43	0
1/10 に希釈	308 ± 21	20

－デルマタン硫酸及びコンドロイチン硫酸の新合成（ＤＳ及びＣＳ）

ラミナイン	Cpm	%取り込み
コントロール0	538 ± 85	-
1/5 に希釈	556 ± 73	-
1/10 に希釈	498 ± 103	-

－ヘパラン硫酸の新合成（ＨＳ）

ラミナイン	Cpm	%取り込み
コントロール0	304 ± 23	-
1/5 に希釈	345 ± 65	-
1/10 に希釈	398 ± 38*	30

[0074]In conclusion, the lamina yne diluted to 1/5 and 1/10 shows a significant operation to new synthesis of the matrix composition component of the Homo sapiens fibroblast in a culture.

[0075]The obtained result actually shows the significant stimulus of new synthesis of the proteoglycans in a film (67%), the film circumference (about 34%), and a matrix (in the case of dilution to one fifth, it is 25%) respectively.

[0076]It became clear that production of the heparan sulfate which exists especially all over the field of a cell membrane was simultaneously stimulated by the quantitative analysis of DS/HS, a DS/CS pair, and HS (it is 30% when it dilutes to 1/10). Existence of PGs in a cell level has main effects in the metabolism of a cell and an extracellular matrix. These [PGs] can cause the interaction of a cell-ligand [of various types], cell-cell, and cell-matrix by those localization.
[0077]The proof fibrocyte of the collagen synthesis stimulus by the fibrocyte in an embodiment 4-culture is wound into a culture under the same condition as Embodiment 3.
[0078]The purpose of the preincubation preincubation of a cell is to synchronize a cell with the G1 period or G0 phase of the cell cycle by reducing the activity of a cell through reduction in a serum factor. After reaching a confluence stage, a culture medium is removed and it transposes to the RPMI culture medium by which 0.5% of FCS was added. Preincubation time is continued for 24 hours.
[0079]After a cell incubation cell incubation finishes, in order to remove a culture medium and to remove the serum component of all the amounts of vestigia, the stratum compactum is rinsed by the RPMI culture medium which does not contain FCS. Incubation operation is performed by the cultivation culture medium which added the vitamin C of the concentration of 0.28 mmole/l, or the lamina yne of various degrees of dilution.
[0080]The concentration of the used vitamin C is ideal for activating not only secretion of collagen but hydroxylation enzyme. By checking lysyl oxidase enzyme, beta-aminopropionitrile of the concentration of 0.2 mmole/l is also added in order to prevent new synthesis collagen depositing in the form of fibril in a cultivation culture medium.
[0081]Cultivation culture media are collected and the stratum compactum is rinsed in the end of incubation time (24 hours).
[0082]the principle amino acids of the quantitative-analysis-procedure of the hydroxyproline which uses HPLC -- OFUTO aldehyde -- being acid (ophtaldehyde acid; OPA) -- it is guided and the interference is excluded. The hydroxyproline and the proline are guided by NBD-Cl by combining an amino group with NBD-Cl. NBD-Hp is separated and identified using negative phase HPLC. In order to prepare separation of an amino acid derivative, the standard containing the hydroxyproline was first combined with NBD-Cl.
- The material hydroxyproline changes in fixed quantity by measuring fluorescence, after dissociating using negative phase HPLC.
- model 2.00 BISHOFU (Bischoff) pump Arcot model (Alcott Model)788 autosampler type automatic injector URUTORASEPU(Ultrasep)C -- 18 column (30 cm x 0.18 cm), A porosity [of 6 micrometers] (porosity)-fluorescence detector, JUSCO (Jasco)821-FI-chromatography conditions : [a mobile phase] ; elution which sets to a part for 1-ml/the; flow rate constituted by the acetonitrile / sodium phosphate buffer mixture, 01 mol/l, and pH 7.2 (9:91 v/v) is performed by stationary mode (stationary mode), and a cycle continues for 10 minutes. A mobile phase is filtered

beforehand and deaerated before use.
- Dissolve preparation NBD-Cl=25mmole of a reagent in methanol.
[0083]It dilutes in order to obtain the solution (50 mg/l) of the hydroxyproline which adjusts 150mmole buffer =0.4 mmole/l;pH into OPQ= methanol 7.2, and the concentration of the range of 0.5 to 40 mg/l.
- Mix the standard solution of concentration with the standard range [l. of 10micro] buffer (pH 9) which is 10microl variously. After adding and agitating OPA of 5microl,; which maintains a tube for 5 minutes with ambient air temperature, next the NBD-Cl solution of 10microl are added. A reaction is performed for 3 minutes at 60 ** under protection from light. And a tube is placed into ice. The obtained coloring is orange and at least 3 hours is stable under protection from light. This mixture 50mul is poured in. A sampling curve is a straight line.
[0084]The sample of a cultivation culture medium is processed similarly and an examination is repeated 3 times.
- Separation and identification of the consequence hydroxyproline were performed using negative phase HPLC. The fluorescence peak was used in order to calculate the hydroxyproline concentration after integration and in a cultivation culture medium.
[0085]
[Table 5]

	ヒドロキシプロリン濃度 mg/ml	増加（%）
コントロール 0	3.67 ± 1.18	-
コントロール＋ビタミンC	5.25 ± 1.31	43
1/5 に希釈	9.72 ± 1.32	164
1/10 に希釈	8.64 ± 0.98	135

[0086]It was admitted the increase in collagen new synthesis was large rather than the lamina yne diluted by 1/5 and 1/10 was guided in confluent Homo sapiens fibrocyte by the vitamin C (43%) used by the optimal concentration for activation of collagen synthesis and hydroxylation enzyme.

[Translation done.]